

# Correspondence

TO THE EDITOR, *British Journal of Venereal Diseases*

## Effect of pH on the motility and virulence of *Treponema pallidum* (Nichols) and *Treponema paraluis-cuniculi* in vitro under anaerobic conditions

Sir,

No published studies have reported the effect of pH on the retention of virulence of *Treponema pallidum*, as measured by rabbit inoculation, as distinct from motility retention in vitro. Using the latter technique, an optimum pH of 7.2 was reported.<sup>1</sup> Furthermore, to the best of our knowledge no in-vitro studies of any kind have been performed with *Treponema paraluis-cuniculi*, the causative agent of rabbit venereal spirochaetoses, and its pH optimum is not known.

We have demonstrated that for both organisms the optimum pH for retention of motility was 7.3-7.6 (figure). However, the more sensitive parameter of virulence retention showed that pH 7.3-7.4 was optimal for both *T. pallidum* and *T. paraluis-cuniculi* (table). Both treponemes were more resistant to alkaline than acidic changes from their optimum pH.

The medium used in our experiments was a slight modification of an anaerobic medium with low redox potential.<sup>2</sup> The experimental pH variants were obtained by adding a few drops of 0.1 mol/l NaOH or 0.1 mol/l HCl to the final medium. Very little was required as the medium was poorly buffered with phosphate. Freshly harvested *T. pallidum* (or *T. paraluis*

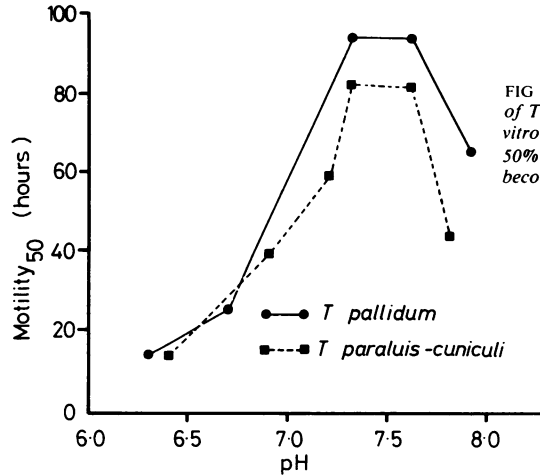


FIG Effect of pH on motility retention of *T. pallidum* and *T. paraluis-cuniculi* in vitro as measured by the time taken for 50% of the treponemes in culture to become immotile (motility<sub>50</sub>).

*cuniculi*) obtained from the orchitic testes of rabbits inoculated with *T. pallidum* two weeks previously (or *T. paraluis cuniculi* four weeks previously) were at a concentration of approximately 10<sup>7</sup> treponemes/ml, and 0.5-ml volumes of these preparations were added to 10 ml of medium under anaerobic conditions. The pH and redox potentials were measured daily to ensure that no changes had occurred. The pH variant media were all isotonic for sheep erythrocytes. Incubation temperature was 34°C under anaerobic conditions. Determinations of treponemal motility and virulence were performed at 24-hour intervals, the former by darkfield microscopical examination of 200 treponemes from duplicate culture tubes and the latter by the quadruplicate inoculation of 0.1 ml of

culture intradermally into the shaved backs of rabbits. The rabbits were kept shaved, cool (18°C), and examined daily for the development of syphilitic lesions until no further lesions appeared.

In view of our findings, we recommend that further studies on the growth in vitro of virulent *T. pallidum* and *T. paraluis-cuniculi* should be carried out at pH 7.3-7.4.

We wish to thank Mr Ian McLean for his excellent technical assistance. The *T. paraluis-cuniculi* (strain 8816) was kindly supplied by Dr A Balows (Centre for Disease Control, Atlanta).

The work was supported by the National Health and Medical Research Council (Australia), the Utah Foundation, the Potter Foundation, the Danks Trust, the estate of the late George Adams and Monash University, from whom financial assistance is gratefully acknowledged.

Yours faithfully,

S Graves

T Gotv

F Trewartha

Department of Microbiology,  
Monash University,  
Victoria, Australia

## References

1. Metzger M, Smogor W. Study of the effect of pH and Eh values of the Nelsen-Diesendruck medium on the survival of virulent *Treponema pallidum*. *Arch Immunol Therap Exp* 1966;14:445-53.
2. Graves S, Sandok P, Jenkin H, Johnson R. Retention of motility and virulence of *Treponema pallidum* (Nichols strain) in vitro. *Infect Immun* 1975;12:1116-20.

TABLE Effect of pH on survival of virulent *T. pallidum* and *T. paraluis-cuniculi* in vitro as measured by latent period of infection in the rabbit

<i>Incubation period (days)</i>	<i>Latent period* of infection (days) after in-vitro incubation at defined pH</i>					
<i>T pallidum</i>	6.3	6.7	7.3	7.6	7.9	
0	7	7	7	7	7	
1	+	13	7	7	7	
2	+	20	11	12	12	
3	+	+	12	12	15	
4	+	+	12	18 (2/4+)	+	
<i>T paraluis-cuniculi</i>	6.4	6.9	7.2	7.4	7.6	7.8
0	14	12	12	14	15	15
1	+	18	17	18	18	18
2	+	+	22 (1/4+)	19	27	35 (1/4)+
3	+	+	+	30 (2/4+)	+	+

\* Period (in days) between rabbit inoculation and appearance of syphilitic lesions as indicated by induration of the skin.  
+ No lesions developed from four inoculation sites on rabbit.

± No of inoculation sites that developed into syphilitic lesions compared with total No of inoculation sites. Where no figures in parentheses are given the value is 4/4.